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Management of Stem-rot of Groundnut (*Arachis hypogaea* L.) Cultivar in Field

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Abstract

The present experiment was conducted at University of Pune for biocontrol of soil-borne plant pathogen *Sclerotium rolfsii* by incorporating arbuscular mycorrhizal fungi (*Glomus fasciculatum*) and conventional system of cultivation with different spacing pattern (15 and 30 cm) in field. Both mycorrhizal inoculation and 30 cm spacing pattern significantly increased growth and yield as compared to control or 15 cm spacing pattern. The pathogenic mycorrhizal groundnut plants in 30 as well as 15 cm spacing pattern showed better growth in terms of plant height, leaf and pod number, fresh and dry weight of whole groundnut plant in comparison to non-mycorrhizal pathogenic ones and the plant growth was better in 30 spacing than 15 cm. The colonization by AM fungi in both spacing pattern was higher in absence of pathogen *S. rolfsii*. However, pathogen's presence decreased the mycorrhizal colonization considerably in 30 and 15 cm. The disease severity and incidence were recorded to be lowered when inoculated with mycorrhiza in pathogenic groundnut plants as compared to non-mycorrhizal pathogenic ones in both spacing pattern and incidence and severity was significantly lower in 30 cm as compared to 15 cm. Therefore, it was observed from our results that for management of soil-borne pathogens inoculation of AM fungi and spacing patterns are necessary.

Keywords: arbuscular mycorrhizal fungi, biocontrol, *Glomus fasciculatum, Sclerotium rolfsii*

Introduction

Groundnut (*Arachis hypogaea* L.) is very important crop of developing countries which contributes around 95% of world production (Nautiyal, 2002). In India it is considered to be an important crop as oilseed crop. The annual production of oil from it is estimated to be in 8 million tons (Patil, 2009). In India, the state of Maharashtra is among chief producer of groundnut crop in the country. However, groundnut production in Maharashtra is not increasing annually as compared to previous years. Also, throughout the world, in comparison to average yield of 825 kg/ha, developing countries contributes much lower than developed countries where yield is 2650 kg/ha. As groundnut is susceptible to wide range of microorganisms which may include fungi, viruses, mycoplasma, nematodes and bacteria, it may have contributed in overall yield losses. *S. rolfsii* Sacc. (teleomorph *Athelia rolfsii*) is among those fungal soil-borne root pathogens which causes severe stem-rot in groundnut plant. There are reports that AM colonization of root systems helps in reducing disease severity caused by soil-borne pathogens such as *Sclerotium, Rhizoctonia, Fusarium* or *Pythium* (Mulongoy *et al.*, 1992; Azcón-Aguilar and Barea, 1996), known to be phenomenon of "bioprotection". Bioprotection not only ensures disease resistance but also helps in development of eco-friendly environment for sustainable agricultural practices today, as chemical measures against soil-borne fungi is often considered to be hazardous and costly. Encouraging results have been well documented by various workers on arbuscular mycorrhizal fungi (AMF) and root-pathogen interactions and prospective of using AMF in biocontrol of many serious soil-borne plant pathogens. In AM association various mechanisms are employed for biocontrol of plant pathogens such as improvement in nutritional status of the host plant (Karagiannidis *et al.*, 2002), competition for host photosynthates (Xavier and Boyetchko 2004), competition for infection/colonization sites (Azcón-Aguilar and Barea, 1996), morphological changes in the roots system (Gutjahr *et al.*, 2009), root damage compensation (Singh *et al.*, 2000), microbial changes in the mycorhizosphere (Li *et al.,* 2007) and activation of plant defence mechanism (Garcia-Garrido and Ocampo, 2002).

Thus, in present investigation *G. fasciculatum* was evaluated for its efficacy against *S. rolfsii* in two field experiments using local groundnut cultivar named 'Phule Pragati (JL-24)'. The aim was to study the effect of mycorrhizal inoculation on seeds of groundnut and disease caused by *S. rolfsii* in field as well as their physiological aspects, growth and yield in cropping system of different plant spacing system of 30 and 15 cm.

Materials and methods

Plant material

In this study the seeds of groundnut (*A. hypogaea* L.) were of local cultivar named 'Phule Pragati (JL-24)' obtained from Naik seeds, Maharashtra, Pune, India. The surface of seeds were sterilized with 0.02% $HgCl_2$ for 5 min and washed thrice with sterile distilled water for planting the seeds in field.

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Isolation, identification and inoculum preparation of AM

Wet sieving and decanting methods according to Gerdemann and Nicolson (1963) was followed for isolation of AM fungal spores. The identification of arbuscular mycorrhiza was carried out by keys suggested by Trappe (1982) and Schenck and Perez (1987). Arbuscular mycorrhizal (AM) fungal spores were determined for colour, shape and dimensions of according to Kornerup and Wanscher (1983). The AM fungi *G. fasciculatum* (Thaxter Sensu Gerd.) Gerd. and Trappe was mass multiplied and maintained in pot cultures for three months with suitable hosts such as *Sorghum vulgare* and *Panicum maximum* (Jacq.) roots grown on 30 cm earthen pots containing 10-15 kg of sterilized soil and sand in a proportion of 1:1.

Twenty grams of mycorrhizal inoculum of *G. fasciculatum* mixture which contained spores, colonized root pieces and extrametrical mycelium in rhizospheric soil was placed at about 3-5 cm below each groundnut seeds under the soil surface before sowing in field.

Pathogen inoculum

S. rolfsii was isolated from basal region of groundnut plants showing stem-rot symptoms from Pune district. Pure culture of *S. rolfsii* was obtained by sub-culturing with the help of single hyphal tip method and was maintained on potato dextrose agar (PDA) slants in incubator at 28°C. *S. rolfsii* was multiplied on sorghum grains (250 g) soaked overnight in water for field experiment. About 100 g of soaked sorghum grains were taken in 500 ml capacity saline bottles tightly plugged. The bottles were then sterilized for 20 min at 121°C. After sterilization the sorghum seeds in saline bottles were inoculated with 5 mm mycelial disc from 7-day-old pure culture of *S. rolfsii* at each bottle and bottles were incubated for a month at 28°C ± 2°C for proper mycelial growth. The grain culture served as pathogen inoculum along with 10 sclerotia of pathogen which were also added to the soil.

Field preparation for experiment

The field was ploughed to a depth of 20 to 25 cm, shape was of in-furrow system and was given ridge with height of about 7-10 cm, inter-row distance 15 cm and plant spacing was 15 cm and 30 cm on rows (16 foot length), and the seeds of groundnut were hand-planted. After three months of mycorrhizal (*G. fasciculatum*) inoculations was made with 20 g of inoculum mixture containing spores and colonized root pieces per 100 g soil (obtained from the culture) and were placed at about 3-5 cm below seeds under the soil surface before sowing.

Planting, growth conditions and experimental design

The experiment consisted of twelve rows in triplicate with four treatments, each one with three replicates in terms of rows with length (16 m) and breadth (10 m) with an area of about 160.00 square meters. Water was supplied at alternate days through irrigation and weeds were removed by hand with the help of hand shovel. No pesticides or fertilizers were applied before or after seeds were sown into rows. All the other agricultural practices were carried out as usual.

Inoculation technique

For the pathogen inoculation soil around the roots was carefully removed without damaging the root. The fungal inoculum of *S. rolfsii* multiplied on Sorghum grains was applied at the rate of 5 g around roots and soil around the roots was replaced by an equal amount of sterile soil to control treatments.

Plant treatments

Complete Randomized Block Design (CRBD) was used. Accordingly, four experimental treatments were allocated:

Controls (C) were without any inoculations or treatments at any particular interval of time.

Inoculation with *G. fasciculatum* (Gf), groundnut plants were inoculated with 20 g of *G. fasciculatum* inoculum below seeds (3-5 cm) before sowing.

Inoculation with pathogen *S. rolfsii* (C+Sr), groundnut plants were inoculated with 5 g of pathogen inoculum (*S. rolfsii*) after fifteen days of plant growth.

Dual inoculations with *G. fasciculatum* and *S. rolfsii* (Gf+Sr), groundnut plants were inoculated with *G. fasciculatum* before sowing and pathogen inoculum was applied after fifteen days of plant growth.

Data collection

Triplicates of plants in each plot were randomly chosen as sample after 30, 60 and 90 days of AM treatment and 15, 45 and 75 days of *S. rolfsii* inoculations.

Parameters observed

Groundnut plants were uprooted to determine number of leaves, plant height, pod number, fresh weight, dry weight, total biomass content and final yield of plants. The total chlorophyll content of the shoot was estimated according to Arnon (1949), Arbuscule percentage (%) and AM colonization was determined by (Phillips and Hayman, 1970).

Disease incidence

Percentage of disease incidence (Kokalis-Burelle *et al*., 1992) was measured using formula:

$$Disease\ incidence\,(\%) = \frac{No.\ of\ infected\ plants}{Total\ no.\ of\ plants} \times 100$$

Disease severity

Disease severity (Ds) was evaluated according to Filion *et al.* (2003) using a rating scale 1-5 as per Shokes *et al.* (1996) (Tab. 1).

Tab. 1. Rating scale for stem rot assessment (Shokes *et al.*, 1996)

Disease rating	Description
1	Healthy
2	Lesions on stem only
3	Up to 25% of the plant symptomic (wilt, dead or dying)
4	26-50% of the plant symptomic
5	>50% of the plant symptomic

Disease severity = Σ (ab) × 100 / AK

Where:

a = No. of diseased plants having the same degree of infection

b = Degree of infection

A = Total no. of examined plants

K = Highest degree of infection

Pathogenicity test

For pathogenicity test of *S. rolfsii*, groundnut seedlings were inoculated with Sorghum seed pathogen inoculum technique in the greenhouse which resulted in at least one diseased seedling or plant with the typical symptoms of the stem-rot disease. The fungus was re-isolated on PDA and colony characteristics were recorded and compared to the original isolates to fulfil Koch's postulates.

Determination of root colonization

Randomly selected root samples were cleared in 10% KOH at 90°C for 1 hour and stained in 0.01% trypan blue (Phillips and Hayman, 1970) for 10 minutes. The fungal structures were visualized under a compound microscope and the measurement of root colonization by *G. fasciculatum* were determined by Grid-line intersect method (Giovannetti and Mosse, 1980).

Mycorrhizal dependency

Mycorrhizal dependency was determined by the dry weights of the plants after drying the tissue to constant weight at 70°C for 48 hours as per method described by (Plenchette *et al.*, 1983).

Production loss

Production losses were calculated using the equation given by Teng (1985):

PL = (AY/1.0-PL) – AY

Where:

PL = Production Loss

AY = Actual yield

Statistical analysis

The experiment was laid in randomized complete block design (CRBD) with three replicates. Data were expressed as mean value of three replicates. The data were subjected to statistical scrutiny following one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). The values are mean ± SD. Duncan's multiple range test was applied as post hoc test at p=0.05. All the calculations were made by using a Statistical Package for Social Sciences (SPSS) for windows version 9.0 and Microsoft Excel 2007 to analyze the data.

Results

Pathogenicity test

Isolate of *S. rolfsii* caused stem rot and wilt in the tested groundnut plants in these experiments. When groundnut seeds were planted on soil and inoculated with *S. rolfsii* symptoms like damping-off and stem rot occurred. Some of the plants were upright in position which showed symptoms of stem rot. The disease was more severe in plants where mycorrhiza was not inoculated. *S. rolfsii* were re-isolated from the seedling of groundnut and the PDA cultures were identical to original isolate.

Disease incidences

The incidences of stem rot disease caused by *S. rolfsii* got reduced due to inoculation by *G. fasciculatum* along with spacing of 30 cm. The disease incidence in 30 cm spacing after 30, 60 and 90 days after AM inoculation and 15, 45 and 75 days after *S. rolfsii* infection was 50.00%, 63.33%, 76.67% respectively for diseased mycorrhizal inoculated groundnut plants (C+Sr) as compared to 36.67%, 40.00%, 53.33% in pathogenic non-mycorrhizal (Gf+Sr) control groundnut plants (Tab. 2). In 15 cm spacing pattern the incidences of stem rot disease caused by *S. rolfsii* was 33.33%, 46.66%, 60% respectively in pathogenic mycorrhizal groundnut plants (Gf+Sr) as compared to pathogen infected non-mycorrhizal control groundnut plants by 53.33%, 70%, 83.33% in (C+Sr) after 30, 60 and 90 days of sowing respectively (Tab. 2). The overall incidence of stem rot caused by *S. rolfsii* got reduced considerably with the inoculation of mycorrhizal fungi *G. fasciculatum* along with 30 cm spacing in particular as compared to 15 cm in groundnut plants.

Disease severity

The results showed that *G. fasciculatum* inoculation and 30 cm over 15 cm spacing on seeds of groundnut significantly reduced stem rot disease severity caused by *S. rolfsii*. The disease severity in 30 cm spacing was 50.00% after 30 days, 36.84% after 60 days and 37.68% after 90 days in pathogen infected non-mycorrhizal groundnut plants (C+Sr), whereas, it was 40.91% after 30 days, 30.30% after 60 days and 28.13% after 90 days in pathogen infected mycorrhizal groundnut plants (Gf+Sr) (Tab. 3). The disease severity was found to be lowered by 33.33%, 28.57% and 29.63% in pathogen infected mycorrhizal treated groundnut plants (Gf+Sr) as compared to non- mycorrhizal pathogen infected groundnut plants by 37.50%, 39.29% and 36.00% in (C+Sr) after 30, 60 and 90 days re-

Tab. 2. Disease incidence (%) and dead plants in *A. hypogaea* L. after 15, 45 and 75 days of *S. rolfsii* inoculation in 30 and 15 cm plant spacing

Spacing	Treatments	Disease incidence (%)			Dead plants (no.)
		15 DAI	45 DAI	75 DAI	75 DAI
30 cm	C	0.00	0.00	0.00	0
	Gf	0.00	0.00	0.00	0
	C+Sr	50.00	63.33	76.67	11
	Gf+Sr	36.67	40.00	53.33	6
15 cm	C	0.00	0.00	0.00	0
	Gf	0.00	0.00	0.00	0
	C+Sr	53.33	70.00	83.33	13
	Gf+Sr	33.33	46.66	60.00	7

C: Control; C+Sr: Control+*S. rolfsii*; Gf: *G. fasciculatum*; Gf+Sr: *G. fasciculatum*+*S. rolfsii*; DAI = Days after inoculation (n=3)

spectively of sowing (15 cm) under field condition. So, to control disease caused pathogen *S. rolfsii,* combination of *G. fasciculatum* inoculation and 30 cm spacing was found to effective than 15 cm (Tab. 3).

Growth responses of groundnut plant

Data obtained from the present investigation revealed that collectively the cropping system and *G. fasciculatum* inoculation on groundnut plants enhanced overall growth. The shoot length, fresh and dry weight and number of pods were significantly higher in the cropping system of 30 cm interspacing than 15 cm interspacing. The growth parameters for both spacing pattern (30 and 15 cm) after 90 days of sowing the number of leaves in mycorrhizal inoculated groundnut plants were significantly higher than non-mycorrhizal control ones. The lowest number of leaves was observed in pathogenic non-mycorrhizal groundnut plants after 90 days of sowing. But mycorrhiza alongwith pathogen showed higher number of leaves as compared to non-mycorrhizal pathogenic ones. The plant height was significantly highest in mycorrhizal treated groundnut plant than non-mycorrhizal groundnut plants. Lowest plant height was observed in non-mycorrhizal groundnut plants infected with pathogen *S. rolfsii*. Plant height was observed to be significantly higher in mycorrhizal groundnut plants infected with *S. rolfsii* as compared to non-mycorrhizal pathogenic ones. Similarly, the number of pods was significantly highest in mycorrhizal groundnut plants whereas lowest pod number was observed in diseased groundnut plants due to *S. rolfsii*. The fresh and dry weight was also significantly highest in mycorrhizal groundnut plant than non-mycorrhizal control ones. The fresh weight was higher in pathogen infected mycorrhizal groundnut plants than pathogenic non-mycorrhizal groundnut plants which was the lowest observation.

For both spacing pattern (30 and 15 cm) the content of total chlorophyll was higher due to increased number of leafs due to mycorrhizal inoculation. The total chlorophyll content significantly increased after 30, 60 and 90 days of sowing in presence of pathogen in mycorrhizal plants as compared to non-mycorrhizal pathogenic groundnut plants. The total chlorophyll content was highest in only mycorrhizal treated groundnut plants, whereas, it was observed to be lowest in diseased groundnut plants due to pathogen *S. rolfsii* (Tab. 4).

Mycorrhizal colonization in groundnut

The percent root colonization for 15 cm spacing was highest in groundnut plants inoculated with *G. fasciculatum* only (Gf) which was observed to be highest by about 88% as compared to pathogen infected mycorrhizal groundnut plants (41% in Gf+Sr) after 90 days of AM inoculation and 75 days after *S. rolfsii* infection. No colonization was observed in non-mycorrhizal (C) or pathogen infected non-mycorrhizal (C+Sr) groundnut plants. The arbuscule percentage in 15 cm spacing was more in

Tab. 3. Disease severity (%) in *A. hypogaea* L. after 15, 45 and 75 days of *S. rolfsii* inoculation in 30 and 15 cm plant spacing

Spacing	Treatments	Disease severity (%)		
		15 DAI	45 DAI	75 DAI
30 cm	C	0.00	0.00	0.00
	Gf	0.00	0.00	0.00
	C+Sr	50.00	36.84	37.68
	Gf+Sr	40.91	30.3	28.13
15 cm	C	0.00	0.00	0.00
	Gf	0.00	0.00	0.00
	C+Sr	37.50	39.29	36.00
	Gf+Sr	33.33	28.57	29.63

C: Control; C+Sr: Control+*S. rolfsii*; Gf: *G. fasciculatum*; Gf+Sr: *G. fasciculatum*+*S. rolfsii*; DAI = Days after inoculation (n=3)

Tab. 4. Leaf number, shoot length, fresh and dry weight of *A. hypogaea* L. after 90 days after sowing and of AM inoculation and 75 days of *S. rolfsii* treatment in 30 and 15 cm plant spacing

Spacing pattern	Treatments	Leaf number (no.)	Shoot length (cm)	Fresh weight (g)	Dry weight (g)
30 cm	C	296.33±31.20c	32.75±1.47bc	61.38±04.71b	28.04±1.70c
	C+Sr	236.66±26.28c	30.25±0.82c	30.56±04.17c	16.53±2.12c
	Gf	589.00±41.88a	40.00±3.80a	143.64±14.39a	66.71±8.39a
	Gf+Sr	469.00±9.89b	37.75±1.92b	79.92±09.28b	45.80±8.23b
15 cm	C	266.00±29.06c	30.00±0.82bc	41.38±4.72bc	24.81±3.28b
	C+Sr	207.66±27.35d	28.00±0.82c	27.23±1.33c	13.53±0.98c
	Gf	514.33±26.61a	38.33±2.87a	144±316.64a	47.37±6.49a
	Gf+Sr	366.67±9.98b	32.67±2.05b	49.92±6.62b	29.13±1.36b

C: Control; C+Sr: Control+*S. rolfsii*; Gf: *G. fasciculatum*; Gf+Sr: *G. fasciculatum*+*S. rolfsii*; Different alphabet (s) in same row represents significance difference at p=0.05 as per Duncan's multiple range test, ± = Standard deviation of the mean; values are mean of three replications; (n=3)

only mycorrhizal treated groundnut plants as compared to pathogen infected groundnut plants (Tab. 5).

The percent root colonization in 30 cm spacing was highest in only mycorrhizal (Gf) groundnut plants by about 87.33% as compared to pathogen infected mycorrhizal groundnut plants (Gf+Sr) after 90 days of AM inoculation and 75 days after *S. rolfsii* infection. Lower colonization was observed in pathogen infected mycorrhizal groundnut plants. The percentage of arbuscule in 30 cm spacing for pathogenic mycorrhizal plants was less as compared to non-pathogenic mycorrhizal groundnut plants. The colonization was appeared to be negligible in non-mycorrhizal or diseased non-mycorrhizal groundnut plants in both spacing pattern (Tab. 6).

Mycorrhizal dependency

The mycorrhizal dependency after 90 days of sowing was 57.62% for only mycorrhizal treated (Gf) and 62.35% for mycorrhizal groundnut plants infected with *S. rolfsii* (Gf+Sr) which was higher in 30 cm spacing pattern. The mycorrhizal dependency in 15 cm spacing for mycorrhizal groundnut plants infected with *S. rolfsii* was higher by 53.35% and for non-pathogenic mycorrhizal groundnut plants (Gf+Sr) it was 46.07% (Tab. 5-6).

Total biomass

In 30 cm spacing pattern increase in total biomass was observed in groundnut plants inoculated with mycorrhiza as compared to non-mycorrhizal control or infected groundnut plants. The total biomass was highest in only mycorrhizal (Gf) treatment by 208.92 gms when compared to non-mycorrhizal control ones (84.46 in Control). In non-mycorrhizal infected ones the total biomass was lowest (51.55 in C+Sr) than pathogen infected mycorrhizal groundnut plants (113.87 in Gf+Sr) after 90 days growth (Tab. 5-6).

In 15 cm spacing the total biomass produced in only mycorrhizal treated groundnut plants were 191.68 in Gf which was highest among all treatments as compared to control ones (66.19 for Control). Mycorrhizal groundnut plants with pathogen infection showed higher total biomass (79.05 in Gf+Sr) as compared to pathogen infected non-mycorrhizal groundnut plants (40.76 in C+Sr) (Tab. 5-6).

Production loss

The production loss was 94.54 g/14.864 m^2 which lower when groundnut plants were planted with spacing of 30 cm as compared to 107.44 g/14.864 m^2 for 15 cm spacing irrespective of inoculation with mycorrhizal fungi *G. fasciculatum* (Fig. 1).

Discussion

The field investigation carried out showed that when groundnut plants were planted very much together observed more disease severity and disease incidence of stem rot or wilting. When groundnut plants were planted closer

Tab. 5. Total biomass, pod number, arbuscule percentage, root colonization and mycorrhizal dependency in *A. hypogaea* L. after 90 days of AM inoculation and 75 days of *S. rolfsii* treatment in 30 cm plant spacing

30 cm Spacing				
Treatments	C	C+Sr	Gf	Gf+Sr
Total plant biomass (g)	84.46	51.55	208.92	113.87
Pod number (no.)	28.00	22.50	37.25	29.50
Arbuscule percentage (%)	0.00	0.00	52.00	12.00
Root colonization (%)	0.00	0.00	87.33	43.00
Mycorrhizal dependency (%)	0.00	0.00	57.62	62.35

C: Control; C+Sr: Control+*S. rolfsii*; Gf: *G. fasciculatum*; Gf+Sr: *G. fasciculatum*+*S. rolfsii*; (n=3)

Tab. 6. Total biomass, pod number, arbuscule percentage, root colonization and mycorrhizal dependency in *A. hypogaea* L. after 90 days of AM inoculation and 75 days of *S. rolfsii* treatment in 15 cm plant spacing

15 cm Spacing				
Treatments	C	C+Sr	Gf	Gf+Sr
Total plant biomass (g)	66.19	40.76	191.68	79.05
Pod number (no.)	24.66	20.00	35.00	27.00
Arbuscule percentage (%)	0.00	0.00	52.00	12.00
Root colonization (%)	0.00	0.00	88.00	41.00
Mycorrhizal dependency (%)	0.00	0.00	46.07	53.35

C: Control; C+Sr: Control+*S. rolfsii*; Gf: *G. fasciculatum*; Gf+Sr: *G. fasciculatum*+*S. rolfsii*; (n=3)

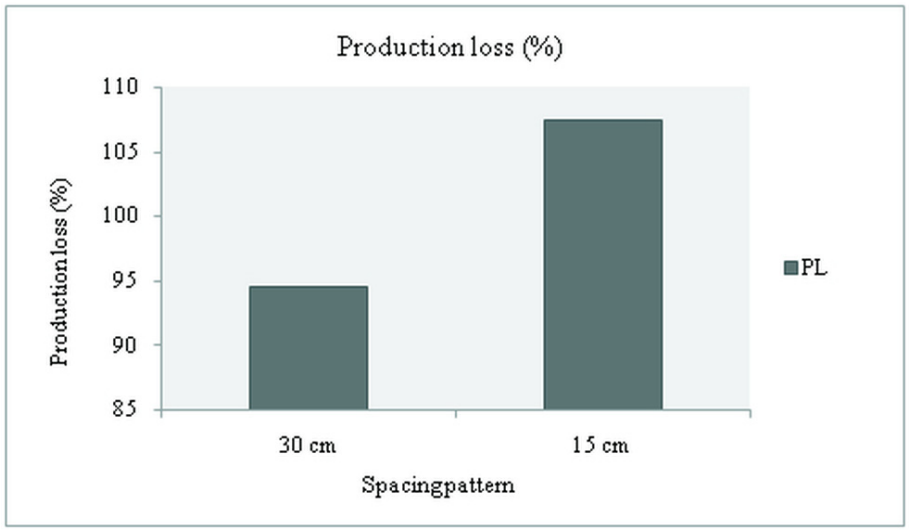

Fig. 1. Production loss (%) in *A. hypogaea* L. after 30, 60 and 90 days of AM inoculation and 75 days of *S. rolfsii* treatment in 30 cm and 15 cm plant spacing

Values are mean of three replications; PL = Production loss; (n=3)

with each other resulted into more plant-to-plant contact which may have led to spread of *S. rolfsii*. Thus, affecting more number of groundnut plants in present in proximity. The above result revealed that at close plant spacing of 15 cm showed significantly higher disease severity as compared to 30 cm spacing of groundnut plants. Similarly, the incidence of disease also were observed to be significantly lower in 30 cm spacing as compared to 15 cm spacing of groundnut plants after 15, 45 and 75 days of pathogen treatment and after 30, 60 and 90 days of AM inoculation (Tab. 7). The total production loss was more in 15 cm spacing than 30 cm spacing system. Even the pattern of rows also affects the yield significantly, as Baldwin and Hook (1998) found more yield in twin rows over four peanut runner cultivars.

The various studies conducted on plant densities have showed on various effects diseases with their respective hosts. The present results are in concurrence with the results of Sconyers *et al*. (2005) where he undertook two microplot studies with peanut (*A. hypogaea* L.) in 2000, 2001 and 2002 to determine the effects of plant spacing, inoculation date and cultivar on stem rot development caused by pathogen *S. rolfsii*, where he found decrease in stem rot severity and incidences with increase in 5-cm increments from 5 to 30. Butzler *et al.* (1998) stated that plant-to-plant infection of Sclerotinia blight of peanut (*Sclerotinia minor* Jagger) occurs after canopy closure and concluded that further spread of the disease could successfully be reduced by mowing excess foliage after disease pressure was evident. By the introduction of mycorrhizal fungi the incidences of disease was also lower in mycorrhizal treated groundnut plant than there non-mycorrhizal pathogenic plants. The reduction in disease by increased distance between plants may be comparable to results found by Sorensen *et al.* (2004). The comparison of spacing shows noticeably some involvement of spacing pattern that reduced the disease severity as well as disease incidences in groundnut plants. One assumption of "critical mass" of the fungal growth was obtained by having several infected plants in close proximity. Irrespective of mechanism the effect on disease severity and incidences were intense.

Tab. 7. Total chlorophyll content in *A. hypogaea* L. after 30, 60 and 90 days of AM inoculation and 75 days of *S. rolfsii* treatment in 30 cm and 15 cm plant spacing

Spacing	Treatments	Total chlorophyll content mg chl./gm of fresh weight		
		30 DAS	60 DAS	90 DAS
30 cm	C	0.457±0.028b	0.487±0.063b	0.676±0.024b
	C+Sr	0.210±0.006c	0.262±0.013c	0.373±0.014c
	Gf	0.562±0.021a	0.764±0.030a	0.864±0.016a
	Gf+Sr	0.495±0.022b	0.497±0.036b	0.695±0.055b
15 cm	C	0.361±0.028b	0.504±0.029b	0.720±0.017b
	C+Sr	0.190±0.009c	0.250±0.015c	0.363±0.011c
	Gf	0.631±0.020a	0.815±0.019a	0.948±0.024a
	Gf+Sr	0.368±0.017b	0.500±0.011b	0.686±0.023b

C: Control; C+Sr: Control+*S. rolfsii*; Gf: *G. fasciculatum*; Gf+Sr: *G. fasciculatum*+*S. rolfsii*; Different alphabet (s) in same row represents significance difference at p=0.05 as per Duncan's multiple range test, ± = Standard deviation of the mean; values are mean of three replications; DAS = Days after sowing; (n=3)

It was not known whether the density had solely affected the disease severity and incidences but the data obtained was in relation to mycorrhiza also. As there is ample evidences available on mycorrhizal fungi and plant pathogen interaction on various hosts (Dehne, 1982; Krishna and Bagyaraj, 1983; Caron *et al.*, 1986a, b, c; Trotta, 1996; Pozo, 2002). Since, vesicular arbuscular mycorrhiza (VAM) were able establish themselves on root of groundnut plants, it can reduced the stem rot disease and root rot disease Krishna and Bagyaraj (1983). Here in this study not only spacing but also the inoculation of mycorrhizal fungi *G. fasciculatum* led to decrease in stem rot severity, disease incidence caused by *S. rolfsii*.

In the study the plant growth was significantly higher in mycorrhizal plants when compared to non-mycorrhizal plant as mycorrhiza increase nutrient flow of host plants (Smith and Read, 1997). The growth response was also higher in pathogenic mycorrhizal groundnut plants as compared to non-mycorrhizal pathogenic groundnut plants as mycorrhizal plants provides stronger vascular system by increase in nutrient flow, mechanical strength and reduction of pathogens effect such as severity and incidence of disease (Schonbeck, 1979). Similar type of observation has been made for host-VAM fungus-pathogen combinations (Schenck *et al.*, 1975). In fact an association can be made between disease severity and incidence by the pathogen and colonization by VAM fungi. The decrease in disease incidences and severity was associated with overall improved growth response of groundnut plants inoculated with mycorrhiza. This benefit was correlated with mycorrhizal dependency (MD) of the mycorrhizal non-pathogenic groundnut plants as compared to pathogenic groundnut plants. During infection by pathogens *S. rolfsii* in groundnut plants the mycorrhizal dependency was significant higher as compare to non-pathogenic mycorrhizal groundnut plants. This suggests the positive benefit of mycorrhizal association during stressed condition than in stress-free condition. Similar kind of observation was made by Declerck *et al.* (2002) on severity in bananas caused by *Cylindrocladium spathiphylli*. There have been many attributed operative mechanism of pathogen suppression by VAM which may be physical, physiological, biological and in biochemical changes. Krishna and Bagyaraj (1983) suggested increase in disease tolerance of groundnut plants inoculated with *G. fasciculatum* and *S. rolfsii* treatment by phosphate nutrition. As mycorrhizal root becomes more lignified to prevent penetration by pathogen (Dehne *et al.*, 1978). Therefore, restricting entry of pathogen into cortex is provided by mycorrhiza (Dehne, 1982). The result revealed various growth responses in terms of significant increase in the leaf number, fresh and dry weight, shoot length, total biomass, pod number and production loss of ground plants due to expansion of the absorptive capacity and their influence in cellular processes (Smith and Gianinazzi-Pearson, 1988) explains increased tolerance of VAM plants towards pathogen (Azcón-Aguilar and Barea, 1996).

The inoculation of mycorrhizal fungi significantly increased the total chlorophyll content in mycorrhizal groundnut plants due to increase in their photosynthetic ability and when mycorrhizal fungus has primary access to photosynthates, the higher carbon demand may inhibit pathogen growth (Azcón-Aguilar and Barea, 1996), thereby causing interference in photosynthetic ability of groundnut plants. But total chlorophyll content was increased in diseased mycorrhizal groundnut plants probably due to colonization by mycorrhiza which helped in increasing photosynthetic ability. As there was more colonization in non-pathogenic mycorrhizal groundnut plants than pathogenic mycorrhizal groundnut plants suggests that this would limit the colonization of the pathogenic fungi to the areas of the root which had not been colonized, thus providing biological protection (Dar *et al.*, 1997). Also it was shown by Cordier *et al.* (1996) that *Phytopthora* does not penetrate arbuscule-containing cells and its development is also reduced in adjacent regions which are showing that even in the absence of systemic resistance, resistance was still induced at some distance from the AM-colonized tissue.

Based on this study, the data verifies the standing problem of stem-rot in field which affects groundnut plants when placing of plants is less than 30 cm and inoculation of mycorrhiza seemed beneficial for overall growth. So, thresholds are needed to characterize the spacing of plants and AM fungi inoculation to reduce stem-rot of groundnut plant.

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